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Cross-Resistance between Azinphos-Methyl and Tebufenozide in the Greenheaded Leafroller, *Planotortrix octo*

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Abstract: Organophosphate(OP)-resistant greenheaded leafroller, *Planotortrix octo*, from Dumbarton, Central Otago, New Zealand, were tested for resistance to tebufenozide and azinphos-methyl. Colonies of *P. octo* were obtained in 1993 and 1995 by tethering virgin females of an OP-susceptible strain (S × S) in apple orchards at Dumbarton, where they mated with wild males, and then raising their progeny (S × D). To remove susceptible insects, first-instar larvae from these colonies were selected respectively four and three times with discriminating doses of azinphos-methyl (1993–94, direct spray) to create colony S × DSe(Az), or tebufenozide (1995–96, diet-sprayed residue) to produce S × DSe(Te).

Dosage mortality tests showed that S × D first-instar larvae were 2- to 4-times resistant to azinphos-methyl and 5- to 8-times resistant to tebufenozide at LD_{50}, compared to S × S. Tests with progeny of isofemale lines of S × D revealed two groups of insects, one 3·5-times resistant and the other 14-times resistant to tebufenozide. After selection, S × DSe(Az) larvae were 14-times resistant to azinphos-methyl and 13-times resistant to tebufenozide, compared to S × S. S × DSe(Te) larvae were 21-times resistant to azinphos-methyl and 76-times resistant to tebufenozide. Resistance of S × DSe(Te) to tebufenozide declined from 269-times at six days to 76-times, 36 days after first exposure. All tests results demonstrated the presence of resistance to azinphos-methyl and tebufenozide in the *P. octo* population and high cross-resistance between these chemicals. Selection with either chemical conferred resistance to the other. Continued use of mating disruption in a resistance management programme at Dumbarton is recommended.

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Key words: tebufenozide; azinphos-methyl; cross-resistance; leafroller; *Planotortrix octo*

1 INTRODUCTION

New Zealand apple orchards are attacked by a complex of five leafroller species (Lepidoptera: Tortricidae), the lightbrown apple moth, *Epiphyas postvittana* (Walker), the greenheaded leafrollers, *Planotortrix octo* Dugdale and *P. excessana* (Walker), and the brownheaded leafrollers, *Ctenopseustis obliquana* (Walker) and *C. herana* (Felder and Rogenhofer). Organophosphates (OPs) have been used for the control of these pests for more than 25 years. However, OP resistance was first reported in *E. postvittana*,[1] and the failure of OP sprays to control leafroller in apple orchards at Dumbarton, Central Otago, was shown to result from OP resistance in *P. octo*.[2] In that study, OP-susceptible virgin female *P. octo* from a colony (S × S) maintained at the Mt Albert Research Centre, Auckland, were tethered and deployed in the Dumbarton apple orchards where they mated

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with wild males. The first-instar larval progeny of these crosses (S × D colony) exhibited 2- to 3-times resistance to azinphos-methyl in a direct spray test in a Potter Tower.[2] This S × D colony could have resulted from crosses with wild males which were resistant, susceptible, or of mixed parentage. Based on the dose-mortality responses of the S × S colony, a discriminating dose of >100 (100–300) mg litre^{-1} azinphos-methyl was applied (using the same Potter Tower method) to this colony to remove susceptible insects and to produce a third colony, S × DSe. Direct spray tests with this colony indicated that resistant insects from Dumbarton were 14- to 20-times resistant to azinphos-methyl, with cross-resistance of 12-times for chlorpyrifos and 8-times for carbaryl.[2]

Tebufenozide (RH5992) has been recently registered (Mimic 70W®, Rohm and Haas Ltd, Philadelphia, USA) in New Zealand for the control of leafrollers and codling moth, *Cydia pomonella* L., in apples. This insecticide belongs to a new class of insect growth regulators, the benzoylhydrazines, which are ecdysone agonists, causing premature apolysis in larvae.[3] Tebufenozide is especially effective against lepidopteran pests of apple,[4] including those in New Zealand.[5]

The S × D and S × DSe colonies of *P. octo* described by Wearing[2] had been maintained for seven generations in the laboratory from April 1993 to June 1994. Resistance tests with tebufenozide were first carried out in July/August 1994 with larvae of the eighth generation. The results of these tests indicated the existence of resistance to tebufenozide in the colonies and cross-resistance to azinphos-methyl. This paper describes these and subsequent tests designed to investigate this phenomenon.

2 METHODS

2.1 Insecticide-susceptible colony of *Planotortrix octo*

The Insect Rearing Unit (IRU) of HortResearch maintains a colony of *P. octo* (S × S) which is known to be susceptible to OP insecticides. The unit supplied eggs from this colony to the Clyde Research Centre where dosage-mortality tests were carried out with first-instar larvae for comparison with the results for the Dumbarton-based colonies (S × D and S × DSe).

2.2 Establishment of S × D colonies of *Planotortrix octo*

The original S × D colonies of *P. octo* were established in 1993 and were re-established in 1995 using the same techniques. Adult female S × S *P. octo* were tethered in the field at Dumbarton. The tethering technique was based on the method of Suckling *et al.*[6] with *E. postvittana* and the same as that used for evaluating the efficacy of mating disruption of *P. octo*.[7] A cotton thread was attached to the forewing of the moth, with the other end stapled to the centre of the base of a Pherocon 1CP trap. The tethered females remained in the field for four to seven days while mating occurred, and were then recovered. The moths were placed in blocks of apples which had been sprayed with a standard OP insecticide programme during the season. In 1993, the moths were deployed from 20 to 29 April and recovered from 26 April to 5 May. A colony was established on artificial diet using larval progeny of 20 females. To re-establish the colonies in April 1995, the same technique provided larvae from 14 tethered females.

The recovered females were returned to the laboratory and placed in individual oviposition cages (polyethylene bags) at 20°C for egg-laying. In 1993, the progeny of these females were bulked to form the S × D colony and were designated S × D1 for the first generation. However, in 1995 the eggs and the larvae which hatched (S × D1) were at first kept separate for each female so that isofemale 'lines' were established for the insecticide testing in the second generation (S × D2). The larvae were reared individually in plastic tubes containing artificial diet with a total founding colony of 800–1000. After the dosage-mortality tests with S × D2, all insects were mixed for continuation of the colony (i.e. isofemale lines were discontinued).

The larvae of the S × D colonies were the product of crosses between insecticide-susceptible females (ex-laboratory) and wild males in Dumbarton. The males may have been either resistant (e.g. RR or RS) or susceptible (e.g. SS), resulting in offspring which could be either susceptible × susceptible or susceptible × resistant.

2.3 Selection for resistance

Unless otherwise stated, the tebufenozide formulation used in the selections and dosage-mortality tests was Mimic70W® and the azinphos-methyl formulation was Gusathion 50WP. Rates of insecticides used in the selections and dosage-mortality tests are presented as active ingredient.

Because of the mixed parentage of the S × D, selection with a discriminating dose of insecticide (azinphos-methyl in 1993–94, tebufenozide in 1995–96) was applied to parts of these colonies to provide a third colony type S × DSe, which would more accurately reflect the resistance level of the wild *P. octo* at Dumbarton. In 1993–94, the selections were carried out four times with azinphos-methyl (S × DSe(Az)) at 100–300 mg litre^{-1} over seven generations as described by Wearing.[2] In 1995, the survivors of some of the higher concentrations of tebufenozide applied to S × D2 in the

dosage-mortality tests (Rohm and Haas Ltd bioassay—see Section 2.4.2), were retained to establish S × DSe(Te). A different method was used for the main (subsequent) selections. A thin layer of artificial diet (3·3–4 g) was spread in the bottom of a standard 9-cm diameter plastic Petri dish and sprayed in a Potter Tower (0·11–0·12 g spray was deposited on the diet). Each spray used 2 ml of a suspension of tebufenozide in tap water applied at 104 kPa (15 psi) with a 10-s settling time. The application temperature was 15–18°C. After spraying, the dish was removed and allowed to dry overnight at about 18°C. On the following day, 30–60 neonate larvae were placed on the diet and the dish was closed. After 10–11 days at 20°C, the surviving larvae were transferred to individual diet tubes and reared through to the next generation.

2.4 Dosage-mortality tests

2.4.1 Dosage-mortality tests with tebufenozide—Method 1

The first method was similar to the main selection procedure (see above), except that only 20–30 neonate larvae (<24 h old) were placed in each dish on the day after it had been sprayed. Neonate larvae were transferred to the diet with a camel hair brush. For spraying, the tebufenozide was suspended in tap water and a dilution series prepared at a range of 9–14 concentrations for each replicated test. The need for this unusually wide range of concentrations arose because of (i) the progressive mortality of the larvae between six and 36 days, and (ii) the objective of providing reliable dosage-mortality lines for six, nine, 18 and 36 days after treatment began. Tap water was used as a control treatment. There were normally 120 larvae per concentration and greater numbers of larvae were used in the 'control'. Larval mortality was recorded six and nine days after the larvae had been placed on the diet and kept at 20°C. Dead larvae were those which failed to move when stimulated gently with a camel-hair brush. On the second occasion, the surviving larvae were transferred to individual diet tubes and retained further to assess mortality after 18 and 36 days at 20°C.

Preliminary tests of this bioassay were conducted in 1994 on S × S and S × DSe(Az)7 using a 200 g kg^{-1} formulation of tebufenozide. The bioassay was then used with Mimic70W® in July/August 1994 for tests on S × S, S × D8 and S × DSe(Az)8. At that time, the S × DSe(Az)8 colony had been selected four times with azinphos-methyl.[2] Mortality assessments were carried out after six days. This method was again used in May/June 1996 for tests on S × S, S × D8 and S × DSe(Te)7. At that time, the S × DSe(Te)7 colony had been selected three times with tebufenozide. Mortality assessments were completed at six, nine, 18 and 36 days.

2.4.2 Dosage-mortality tests with tebufenozide—Method 2

The second method was one developed by Rohm and Haas Ltd. It was used in 1995 for tests on the isofemale lines of colony S × D2 and on S × S. Warm artificial diet (10 ml) was dispensed into plastic Dixie® cups, and after cooling, 0·5 ml of aqueous suspension of tebufenozide was pipetted onto the surface. The treated diet was allowed to dry at 20°C while the insecticide soaked into the surface of the diet. A dye test indicated that this was only into the top 1–2 mm of the diet. A minimum of seven concentrations of tebufenozide and a water-treated control were used for each replicate dosage-mortality test. For each concentration, a minimum of 20 cups were normally used, each containing five first-instar larvae. Neonate larvae <24 h old were transferred to the treated diet with a camel-hair brush and the cups were closed. Larval mortality was recorded after seven, 14 and 21 days. Dead larvae were those which failed to move when stimulated gently with a camel-hair brush.

With the S × S colony, a total of 80 to 180 larvae were used per concentration. With the S × D colony, the results from the replicate dishes for the progeny of each female were first combined to give a total of about 250 larvae per concentration for analysis. The results also indicated differences in survival in the progeny of different females, with Group 1 comprising females with higher survival (153–159 larvae per concentration) and Group 2 comprising females with lower survival (80–100 larvae per concentration).

2.4.3 Dosage-mortality tests with azinphos-methyl

The azinphos-methyl test was described by Wearing.[2] First-instar larvae of *P. octo* were subjected to a direct spray test in a Potter Tower. The azinphos-methyl was suspended in tap water and a dilution series prepared at a range of six to nine concentrations. Tap water was used as a control treatment. Each spray through the Potter Tower used 2 ml of suspension applied at 104 kPa (15 psi) with a 10-s settling time. The application temperature was 15–18°C. Twenty to thirty first-instar larvae (<24 h old) were placed in a standard 9-cm diameter plastic Petri dish and sprayed in the Potter Tower. The larvae were held in the closed dish for 10 min after spraying and then transferred to a similar dish containing a thin layer of artificial diet. This dish was closed and the larvae were then held for 48 h at 20°C until mortality assessment. Larvae were considered dead if no movement was detected in response to gentle manipulation with a camel-hair brush. There were 100–120 larvae per concentration, including the 'control' treatment.

This bioassay with azinphos-methyl was used in 1993–94 and the results were described by Wearing.[2] In May/June 1996, the azinphos-methyl bioassay was used for tests on S × S, S × D8 and S × DSe(Te)7. At that

time, the S × DSe(Te)7 colony had been selected three times with tebufenozide.

2.5 Statistical analysis

For all three bioassay methods, the mortality data were transformed to probits and analysed using Polo-PC,[8] which calculates the regression of probit mortality on the logarithm of the concentration of insecticide. Polo-PC was also used to compare the dosage-response lines for the different colonies, primarily using resistance ratios at LD_{50}. LD values are expressed as the rate of insecticide active ingredient in the suspensions used to spray or treat the insects or diet, and not the rate contained in the diet itself.

3 RESULTS AND DISCUSSION

3.1 Selection programme

3.1.1 Azinphos-methyl selections

A detailed description of the selections of S × D with azinphos-methyl during 1993–94 to produce S × DSe(Az) was provided in Wearing.[2] Four selections were carried out in generations 1, 3, 5 and 6 and the associated average larval mortalities ranged from 82 to 90%.

3.1.2 Tebufenozide selections

Larvae of S × D2 in 1995 which survived the first dosage-mortality test with tebufenozide at 1·4 mg $litre^{-1}$ and 1·9 mg $litre^{-1}$ were retained to establish the S × DSe(Te)2 colony. Larval mortality from this selection was 82·4% ($n = 511$); larval mortality in the two subsequent selections was respectively 93·4% (generation 5, $n = 4886$) and 88·2% (generation 6, $n = 5805$).

3.2 Dosage-mortality tests

3.2.1 Tebufenozide tests with S × DSe(Az)—Method 1 1994

The preliminary dosage-mortality test using tebufenozide 20% formulation in July 1994 provided the first evidence that the S × DSe(Az) colony was resistant to tebufenozide. Mortality data were adequately described by the log probit model for the S × S colony at four, six and nine days (LD_{50} 3·9 mg $litre^{-1}$) but mortality was so low in the S × DSe(Az)7 larvae that dosage-mortality lines were not obtained, even for the nine-day data (LD_{50} 60·2 mg $litre^{-1}$).

A full dosage-mortality test using six concentrations of tebufenozide (Mimic70W®) on S × S, S × D8 and S × DSe(Az)8 confirmed the resistance to tebufenozide of the S × DSe(Az) colony (LD_{50} 39·4 mg $litre^{-1}$) compared to both the unselected S × D colony (6-times at LD_{50}) and the S × S colony (13-times at LD_{50}) (Fig. 1). The resistance factor between S × D8 and S × S was 2·3-fold at LD_{50}. These relationships are very similar to those obtained a few weeks earlier in tests with azinphos-methyl using the same colonies, with resistance factors of 5-, 14- and 2·3-fold respectively.[2] The data showed that selection of S × DSe(Az) with azinphos-methyl had conferred cross-resistance to tebufenozide.

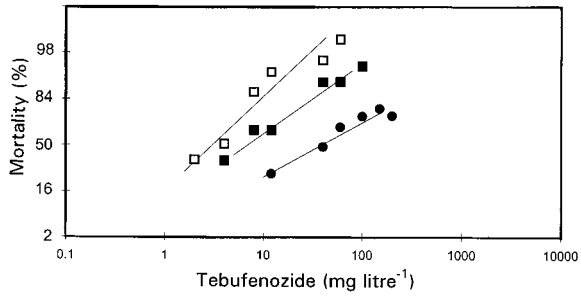

Fig. 1. Response of first-instar larvae of *Planotortrix octo* six days after first exposure to tebufenozide spray deposits on artificial diet. (□) Colony S × S, (■) Colony S × D8, (●) Colony S × DSe(Az)8.

3.2.2 Tebufenozide tests with S × D—Method 2 1995

The second bioassay method for tebufenozide resistance was undertaken to provide a further rigorous test of its presence in the *P. octo* population and the link with azinphos-methyl resistance. The tests were carried out with S × S and S × D2 and the replicates for S × D2 were the progeny of isofemale lines. The results of the seven- and 14-day assessments are summarised respectively in Fig. 2 and Table 1. All the dosage-mortality lines for seven, 14 and 21 days were described adequately by the log probit model.

The dosage-mortality lines for S × D2 and S × S were significantly different after seven days with a resistance factor of 6-fold at LD_{50}. When the progeny of the different females were sorted into those with distinctly higher (Group 1) and lower (Group 2) survival, Group 1 larvae were 11-times resistant to tebufenozide at LD_{50} compared to S × S whereas Group 2 larvae were 3-times resistant (Fig. 2). The LD_{50} values of both S × D2 and S × S declined significantly ($P < 0·05$) from

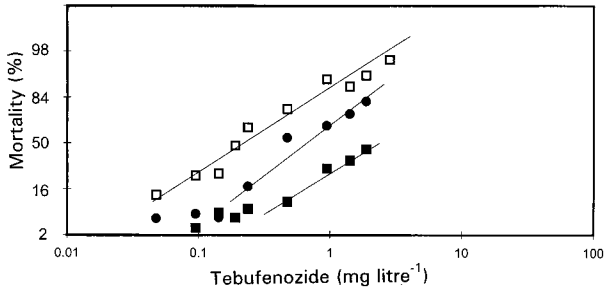

Fig. 2. Response of first-instar larvae of *Planotortrix octo* seven days after first exposure to tebufenozide deposits pipetted onto artificial diet. (□) Colony S × S, (■) Colony S × D8 Group 1, (●) Colony S × D8 Group 2 (see text).

TABLE 1
Responses of S × S *Planotortrix octo* First-Instar Larvae to Artificial Diet Coated with Tebufenozide, Compared to the S × D2 Colony

Colony	*N*	*Slope b*	*Standard error of b*	LD_{50} (*mg litre*$^{-1}$)	*95% CL*
S × S 14 days	1083	2·50	0·20	0·11	0·09–0·13
S × D2 14 days	2046	1·97	0·16	0·89	0·71–1·07
S × D2 Group 1 14 days	1256	2·38	0·42	1·56	1·33–1·87
S × D2 Group 2 14 days	771	2·60	0·23	0·38	0·24–0·53

seven to 14 days and the difference between S × D2 and S × S increased, with a resistance factor of 8-fold at LD_{50} (Table 1). The difference between Group 1 and Group 2 increased from the seven- to the 14-day assessments. Group 1 larvae were 14-times resistant to tebufenozide at LD_{50} compared to S × S whereas Group 2 larvae were 3·5-times resistant.

Isofemale lines were maintained up to the time of the tests because of the possible differences in the resistance traits carried by wild Dumbarton males mating with the tethered females. The value of this procedure was validated by the results, which indicated significant differences between the males which gave rise to the larvae in Groups 1 and 2. The resistance factors (14 days) of the progeny at 3- to 4-fold (S × D2 Group 2) and 14- to 15-fold (S × D2 Group 1) were similar to those obtained in the earlier tests with both tebufenozide and azinphos-methyl using respectively S × D (in which D was a mix of males) and S × DSe(Az) (in which susceptible males had been removed by selection with azinphos-methyl). The current tests confirmed the earlier evidence of resistance to tebufenozide in the Dumbarton *P. octo* and of high cross-resistance between azinphos-methyl and tebufenozide. The information derived from both series of tests (Methods 1 and 2) and Wearing[2] indicated that *P. octo* populations at Dumbarton include a strain which is at least 14-fold resistant to tebufenozide and azinphos-methyl compared to the known OP-susceptible strain.

The subsequent performance of the S × D2 larvae surviving from the bioassay tests showed that there was no clear trend of further decreasing survival as the concentration of tebufenozide increased. There was high percentage adult emergence in the untreated controls (91%) and in all the concentrations (0·05–1·9 mg litre^{-1}) of tebufenozide (73 to 91%). The surviving adult moths of each concentration mated successfully and produced viable eggs, as confirmed by hatching. The fecundity of the moths was not measured.

3.2.3 Tebufenozide tests with S × DSe(Te)—Method 1 1996

The 1996 tests aimed to determine whether discriminating dose selection with tebufenozide would increase resistance to tebufenozide and confer cross-resistance to azinphos-methyl. Examples of the dosage-mortality lines for the S × DSe(Te) tests with tebufenozide are given in Table 2. All the dosage-mortality lines for six, nine, 18 and 36 days were described adequately by the log probit model.

The six-day results for the S × S colony (Table 2) can be compared directly with those obtained in 1994 using the same bioassay (see Fig. 1) and using the same formulation of tebufenozide. There were no significant differences in the LD_{10}, LD_{50} or LD_{90} values in the two tests, with the current LD_{50} at 2·7 mg litre^{-1} compared to 3·0 mg litre^{-1} in 1994.

The six-day results for the S × D colony can be compared in like manner. Although the LD_{10} did not differ between the two tests, the LD_{50} and LD_{90} values in 1996 (16 and 220 mg litre^{-1}) were significantly higher ($P < 0·05$) than those obtained in 1994 (7 and 48 mg litre^{-1} respectively—Fig. 1). This may reflect the small numbers of founding females on each occasion and the varying proportion of resistant males with which they mated, which could influence interpretation of the levels of resistance. As a result of these differences, the S × D8 colony in 1996 was 6-times resistant to tebufenozide at LD_{50} (Table 2) compared to only a 3-fold resistance in the S × D8 colony of 1994 (Fig. 1). This 6-times resistance of S × D8 at six days in 1996 is very similar to the 6-times resistance of S × D2 at seven days found in

TABLE 2
Responses of S × S *Planotortrix octo* First-Instar Larvae to Artificial Diet Sprayed with Tebufenozide Compared to the S × D8 and S × DSe(Te)7 Colonies

Colony	*N*	*Slope b*	*Standard error of b*	LD_{50} (*mg litre*$^{-1}$)	*95% CL*
S × S, 6 days	1172	1·42	0·11	2·7	1·96–3·46
S × D8, 6 days	1266	1·13	0·10	16·04	11·77–21·40
S × DSe(Te)7, 6 days	1527	1·15	0·10	720·53	569·23–965·91
S × S, 36 days	1534	2·19	0·16	0·44	0·33–0·55
S × D8, 36 days	1266	1·11	0·08	1·98	1·10–3·10
S × DSe(Te)7, 36 days	1524	2·18	0·11	33·42	25·91–40·78

1995 with the same S × D colony (Rohm and Haas Ltd bioassay). These results indicate that the resistance level of the 1995 S × D colony did not decline in the absence of selection pressure over the six generations from S × D2 to S × D8.

Tebufenozide is ingested, causing larval mortality for many days after exposure and resulting in falling LD values over that time (Table 3). For this reason the best estimates of resistance are those obtained from the final 36-day assessments, by which time many surviving larvae had pupated (Tables 2 and 3). The results which have just been described for S × D8 showed that the resistance level of this colony at LD_{50} changed little from the six-day (6-times) to the 36-day (4·5-times) assessments (Table 3). However, the S × DSe(Te)7 colony was very different, and an initial resistance factor of 269-fold at six days fell progressively to 76-fold at 36 days (Table 3).

These changes indicated that the larvae of S × DSe(Te) died much more slowly than those of S × S (or indeed S × D), as well as having a high final resistance level. The relative potency between S × DSe(Te)7 and S × D8 fell similarly from 46-times to 10-times. This was the first time that this phenomenon had been observed and it was not seen with the tebufenozide resistance obtained by selection with azinphos-methyl. Three selections with tebufenozide also resulted in much higher levels of resistance (76-times at LD_{50}) to tebufenozide (Tables 2 and 3) than was obtained after four selections using azinphos-methyl (13-times resistance to tebufenozide at LD_{50}—Fig. 1). Larvae were able to survive for six days even when treated with 1960 mg litre^{-1} of tebufenozide.

The slopes of the dosage-mortality lines for S × S and S × DSe(Te)7 increased from the six-day to the 36-day assessments (Table 3), indicating an increasingly homogeneous response to treatment. S × D8 maintained a low slope throughout and because of these slope differences, relative potency between S × D8 and the other colonies provided a better estimate of resistance than LD_{50} comparisons at 18 and 36 days, by giving greater weight to survival at higher concentrations of tebufenozide (Table 3).

3.2.4 Azinphos-methyl tests with S × DSe(Te)—1996

All the dosage-mortality lines (Fig. 3) were described adequately by the log probit model.

The results for the S × S colony (Fig. 3) can be compared directly with those obtained in the most recent previous tests in June 1994 using the same bioassay[2] and the same formulation of azinphos-methyl. There were no significant differences in the LD_{10}, LD_{50} or LD_{90} values in the two tests, with the current LD_{50} at 14·4 mg litre^{-1} compared to 14·6 mg litre^{-1} in 1994.

The results for the S × D colony can be compared in like manner. The LD_{10} (14·3 mg litre^{-1}), LD_{50}

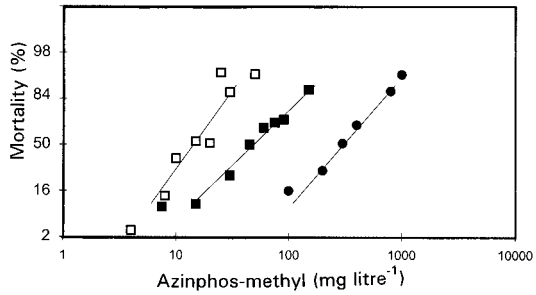

Fig. 3. Response of first-instar larvae of *Planotortrix octo* to direct spraying with azinphos-methyl. (□) Colony S × S, (■) Colony S × D8, (●) Colony S × DSe(Te)7.

TABLE 3

Changes from six to 36 Days in the Responses of S × S *Planotortrix octo* First-Instar Larvae to Artificial Diet Sprayed with Tebufenozide Compared to the S × D8 and S × DSe(Te)7 Colonies

Assessed at (days)	*S × S*		*S × D8*		*S × DSe(Te)7*	
	LD_{50} [a]	*Slope b*	LD_{50} [a]	*Slope b*	LD_{50} [a]	*Slope b*
6	2·7	1·42 ± 0·11	16·0	1·13 ± 0·10	720·5	1·15 ± 0·10
9	0·9	1·16 ± 0·11	5·0	1·26 ± 0·11	111·4	1·37 ± 0·10
18	0·5	2·07 ± 0·14	2·1	1·12 ± 0·08	35·1	2·03 ± 0·15
36	0·4	2·19 ± 0·16	2·0	1·11 ± 0·08	33·4	2·18 ± 0·11

Assessed at (days)	*S × S versus S × D8*		*S × D8 versus S × DSe(Te)7*		*S × S versus S × DSe(Te)7*	
	Resistance factor LD_{50}	*Relative potency*	*Resistance factor* LD_{50}	*Relative potency*	*Resistance factor* LD_{50}	*Relative potency*
6	6·0×	6×	45×	46×	269×	255×
9	5·5×	5×	22×	22×	122×	106×
18	4·6×	7×	17×	11×	78×	80×
36	4·5×	7×	17×	10×	76×	76×

[a] mg litre^{-1}.

(49·3 mg litre^{-1}) and LD_{90} (170 mg litre^{-1}) values for S × D8 in 1996 (Fig. 3) were higher than those obtained in 1994 (10·4, 33·3 and 106·6 mg litre^{-1} respectively),[2] again suggesting that a higher proportion of resistant males, or males with higher resistance, mated with the tethered females in 1995 than in 1993.

The discriminating dose of tebufenozide applied three times to S × DSe(Te) colony during 1995–96 resulted in an increase of azinphos-methyl resistance to 22-times at LC_{50} compared to S × S (Fig. 3). This level of resistance is similar to that obtained after four selections with azinphos-methyl in earlier research[2] (14- to 20-times). The LD_{50} of the S × DSe(Te)7 colony in 1996 (308 mg litre^{-1}) after tebufenozide selection was significantly higher ($P < 0·05$) than the LD_{50} of S × DSe(Az)7 in 1994 (180 mg litre^{-1}) after azinphos-methyl selection. However, when compared to the S × D colony, the three tebufenozide selections (1996) or four azinphos-methyl selections (1994) each increased resistance 6-fold. Whereas selection with azinphos-methyl resulted in similar levels of resistance to both azinphos-methyl and tebufenozide, selection with tebufenozide resulted in higher resistance to tebufenozide than to azinphos-methyl.

3.3 Comparison with other cases of tebufenozide resistance

These tests were carried out after many years of use of azinphos-methyl in the orchards at Dumbarton but before the use of tebufenozide. The results not only confirmed resistance to azinphos-methyl in the *P. octo* population at Dumbarton[2] but also demonstrated the presence of tebufenozide resistance. The results have confirmed cross-resistance between tebufenozide and azinphos-methyl; selection of S × D by either chemical conferred resistance to the other. This is the first known case of such cross-resistance between these chemicals and the first reported case of tebufenozide resistance in a leafroller species.

Other studies with tortricids which have investigated the relationship between OP resistance and tebufenozide have failed to detect cross-resistance (e.g. Biddinger *et al.*[9]). Cross-resistance between azinphos-methyl and diflubenzuron in codling moth has been regularly reported since 1988.[10,11] Sauphanor *et al.*[12] recorded 370-fold resistance to diflubenzuron in codling moth, with cross-resistance to the other benzoylureas, to tebufenozide, and possibly to fenoxycarb.[13] By implication, these combined results suggest a risk of cross-resistance between azinphos-methyl and tebufenozide in codling moth but this has yet to be demonstrated. Tests on OP-resistant strains of codling moth in South Africa and California have shown no cross-resistance to tebufenozide (R. L. Oakes, Rohm and Haas Ltd, pers. comm.) Ishaaya *et al.*[14] reported mild cross-resistance to tebufenozide (3·5-times) in a strain of Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval), with >100-times resistance to cypermethrin.

In the current study with *P. octo*, there was a high level of cross-resistance between azinphos-methyl and tebufenozide following selection with either. These results suggest that a common mechanism(s) is involved in the resistances to the two insecticides. However, while the level of resistance to azinphos-methyl was similar after selection with either chemical, the resistance to tebufenozide was higher after selection with tebufenozide than that after azinphos-methyl selection. This suggests that an additional mechanism(s) may play a role in tebufenozide resistance following tebufenozide selection. Additional mechanisms are also suggested by the long decline in resistance of S × DSe(Te)7 from 269-times assessed at six days to 76-times at 36 days. While most of the mortality from tebufenozide commonly occurred over a period of two to three weeks, this long delay suggests that the insects of S × DSe(Te) (but not S × DSe(Az)) have a metabolic mechanism for disposing of the tebufenozide after ingestion (see e.g. Smagghe *et al.*[15]).

No studies have yet been made of the mechanisms involved in *P. octo* resistance to azinphos-methyl or tebufenozide. Biddinger *et al.*[9] discussed the enzymes potentially involved in cross-resistance of *P. idaeusalis* to OPs and insect growth regulator compounds. Sundaram *et al.*[16] have reported anti-feedant action of tebufenozide on spruce budworm, *Choristoneura fumiferana* Clemens, larvae and this is a possible mechanism which could be involved in resistance.

The differing levels of tebufenozide resistance in the two groups of isofemale lines of *P. octo* in the current work may be related to the mating partners of the tethered females. If a single gene is involved in the resistance, the two groups may have resulted from crosses between SS females and either RS or RR males. However, further research is needed to determine this, as the two groups may be part of a continuum if more resistance genes are involved. Preliminary analyses of the relationships between the dosage-mortality responses of S × S, S × D and S × DSe indicate that resistance may be incompletely recessive. This conclusion is indicated if it is assumed that (i) S × D is entirely RS and did not change composition during laboratory rearing, and (ii) S × DSe is homozygous RR. Under these assumptions, data from Figs 1 and 3, and Table 2 (36 days) give degrees of dominance of −0.34, −0.30 and −0.19 respectively.

3.4 Implications for field control

The loss of field control of leafroller with OP insecticides in apples at Dumbarton prompted the investigation and led to the discovery of resistance.[2] The

resistance management programme which has been operated over the past four seasons at Dumbarton has used mating disruption and has been very effective in reducing leafroller damage, despite the continued use of OPs.[6] Tebufenozide was used extensively in 1996–97 on many commercial apple orchards in Central Otago and gave excellent control (<0.4% damage at harvest); at Dumbarton where mating disruption was used in combination with tebufenozide, damage on different cultivars ranged from 0 to 2.0%.[17]

A feature of the resistance at Dumbarton is its lack of spread from a small number of orchards, despite being present for several years before it was investigated.[2] The abundance of susceptible *P. octo* in the orchard environment was thought to be primarily responsible for this but, if preliminary analysis is confirmed, the lack of spread may have been assisted by the resistance being incompletely recessive. These gene flow and recessive effects are likely to be extremely important in the field population. Unlike laboratory selection, in which resistant moths are permitted to mate only with other resistant moths, field selection is likely to be much slower where there is abundance of susceptible moths in the environment. Provided ecological factors, such as immigration of susceptibles, are maintained, and resistance remains recessive, only slow change should be expected in the resistance of field populations to either OPs or tebufenozide at Dumbarton. Although tebufenozide is a more persistent product, the reduced frequency of spraying and greater selectivity of tebufenozide compared to OPs should assist in further slowing resistance increase.

Other cases of OP resistance in *P. octo* are now being reported from Hawkes Bay in the North Island of New Zealand.[18] It cannot be assumed that the cross-resistance of azinphos-methyl and tebufenozide at Dumbarton also occurs at these new sites. However, it would be prudent to institute similar resistance management procedures until the spectrum of resistance is known.

4 CONCLUSIONS

A population of *P. octo* from Dumbarton, Central Otago, New Zealand, which was known to be resistant to azinphos-methyl, has been shown to be cross-resistant to tebufenozide. Despite the very different modes of action of these insecticides, selection with either chemical conferred resistance to the other. Further research is required to determine the mechanisms of resistance and cross-resistance. The present successful system of resistance management, which uses mating disruption combined with reduced insecticide spraying, is being continued as tebufenozide is introduced to the orchard pest management programmes.

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